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Direct Comparison of Alere i and cobas Liat Influenza A and B Tests for Rapid Detection of Influenza Virus Infection

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We compared two rapid, point-of care nucleic acid amplification tests for detection of influenza A and B viruses (Alere i [Alere] and cobas Liat [Roche Diagnostics]) with the influenza A and B virus test components of the FilmArray respiratory panel (BioFire Diagnostics) using 129 respiratory specimens collected in universal viral transport medium (80 influenza A virus and 16 influenza B virus positive) from both adult and pediatric patients. The sensitivities of the Alere test were 71.3% for influenza A virus and 93.3% for influenza B virus, with specificities of 100% for both viruses. The sensitivities and specificities of the Liat test were 100% for both influenza A and B viruses. The poor sensitivity of the Alere test for detection of influenza A virus was likely due to a study set that included many low-positive samples that were below its limit of detection.

Influenza is a significant cause of morbidity and mortality worldwide. Although the diagnosis is often made by clinical signs and symptoms alone, laboratory testing may be needed to guide antiviral therapy, determine isolation precautions, and provide epidemiologic data, since many different respiratory viruses can cause influenza-like illness. The laboratory diagnosis of influenza has evolved from the use of culture and antigen detection tests to nucleic acid amplification tests that are now considered the new gold standard.

Until recently, point-of-care diagnostic testing has been limited to rapid antigen tests based on chromatographic immunoassay technology designed in simple-to-use formats with results available in <30 min. The chromatographic immunoassays typically have suffered from moderate to low sensitivity; however, recent improvements in test chemistries and instrument readout of results have improved their performance characteristics (1–4). Currently, there are two CLIA-waived, FDA-cleared nucleic acid amplification tests designed to be performed as point-of-care tests by nonlaboratory personnel, the Alere i (Alere, Scarborough, MA) and cobas Liat (Roche Diagnostics, Indianapolis IN) influenza A and B tests. These tests hold promise to significantly improve near-patient diagnostic testing for influenza and may facilitate true practice changes in how clinicians manage these patients.

The Alere test is semiautomated and uses an isothermal nicking enzyme amplification reaction and fluorescently labeled molecular beacons to amplify and detect a region of the polymerase basic protein 2 gene in influenza A virus, a region of the polymerase acid protein gene in influenza B virus, and an internal control in less than 15 min (5). The Alere test is intended to be used for direct nasal swabs (CLIA complexity, waived) and for nasal and nasopharyngeal swabs eluted in viral transport medium (CLIA complexity, moderate). The Alere test has reported sensitivities and specificities of from 80 to 99.3% and from 62.5 to 100%, respectively, for detection of influenza A virus and from 45.2 to 97.6% and 53.6 to 100%, respectively, for detection of influenza B virus (5–9). The comparator assays included viral culture in one study and other nucleic acid amplification tests in the remaining studies.

The Liat test automates and integrates sample purification, nucleic acid amplification, and detection of a region of the matrix gene of influenza A virus, a region of the nonstructural protein gene of influenza B virus, and an internal control using real-time reverse transcription-PCR (RT-PCR) in 20 min. It is intended to be used with nasopharyngeal swabs in universal transport medium. The Liat test has reported sensitivities and specificities of from 97.7 to 99.2% and 99.2 to 100%, respectively, for influenza A virus detection and from 96.9 to 100% and 97.9 to 100%, respectively, for influenza B virus detection (10, 11). The comparator assays in these studies were other nucleic acid amplification tests.

In this study, we compared the performance of these two tests to those of our routine influenza A and B tests that are part of the FilmArray respiratory panel (BioFire Diagnostics, Salt Lake City, UT) using 129 respiratory samples collected in 3 ml of universal viral transport medium. To our knowledge, this is the first published direct comparison of the performance characteristics of the Alere and Liat influenza A and B tests.

MATERIALS AND METHODS

Clinical specimens. A total of 129 respiratory specimens collected in 3 ml of universal viral transport medium (BD Diagnostics, Sparks, MD) were used in the study. The specimen types included 126 nasopharyngeal swabs, 2 nasal aspirates, and 1 bronchoalveolar lavage (BAL) fluid sample. Specimens were collected from 76 adult patients from 19 to 81 years of age and 53 pediatric patients from 1 month to 17 years of age (32.1% were less than 1 year old) seen in a variety of outpatient (99 samples) and inpatient (30 samples) locations. The specimens were collected from 29 December 2015 to 4 April 2016.

Study design. All study samples were tested with the FilmArray test for routine diagnostic purposes, and residual samples were frozen at −70°C for ≤4 weeks prior to testing with the Alere and Liat tests. We selected 77

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TABLE 1 Comparison of Alere and Liat with the FilmArray test for detection of influenza A virus

Alere or Liat result	No. with FilmArray result:		
	Positive	Equivocal	Negative
Alere			
Positive	56	1	0
Negative	21	2	49
Liat			
Positive	77	3	0
Negative	0	0	49

samples that were positive for influenza A virus (56, 2009 H1N1; 10, seasonal H1; 7, H3; and 4, A no subtype), 2 samples that were equivocal for influenza A virus (positive in Flu-A pan1 assay only), 1 sample that was equivocal for 2009 H1N1 (positive in both FluA-H1-pan and FluA-H1-2009 assays), 16 samples that were positive for influenza B virus, and 33 samples that were negative for both viruses by the FilmArray test for the study. These samples were thawed once and retested concurrently with both study tests in batches ranging from 3 to 11 samples by a single medical technologist using a single Alere instrument and a single Liat instrument in our clinical laboratory. The Alere, Liat, and FilmArray tests were performed as previously described (6, 11, 12). The FilmArray test uses nested, multiplex PCR and melting curve analysis for the simultaneous detection and identification of multiple respiratory viral and bacterial nucleic acids in respiratory samples. The influenza A virus panel component uses two pan-influenza A virus assays targeting the matrix and nonstructural protein 1 genes and three assays targeting hemagglutinin (HA) genes (pan-HA1, HA1-2009, and HA3) to detect influenza A virus and to differentiate commonly occurring HA types and subtypes.

A quantitative real-time RT-PCR for influenza A virus based on the Centers for Disease Control protocol (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/) was performed at Ionian Biosciences (San Diego, CA) for some of the discordant samples. Residual samples were extracted with the QIAamp viral RNA minikit (Qiagen, Germantown, MD). The 20-μl PCR mixture included 800 nM influenza A virus primers, 200 nM TaqMan probe, 1× Invitrogen SuperScript III Platinum one-step qRT-PCR mix (ThermoFisher Scientific, Waltham, MA), and 5 μl purified nucleic acid. Cycling conditions were as follows: 50°C for 30 min, 95°C for 2 min, and 60 cycles of 95°C for 15 s and 55°C for 30 s. The assay was calibrated with H1N1 influenza A virus (Solomon) RNA obtained from Virapur, San Diego, CA.

This investigator-initiated study was approved by the Institutional Review Board for Human Research of the Medical University of South Carolina (Pro00052080).

Data analysis. The sensitivities and specificities, with 95% confidence intervals (CI), of the Alere and Liat tests for influenza A and B viruses were determined relative to the FilmArray tests for the same viruses. The results of the Alere and Liat tests were compared directly using the McNemar test. All statistical analyses were performed using Analyze-it for Microsoft Excel version 3.76.1 (Analyze-it Software, Leeds, United Kingdom).

RESULTS

The results of the three nucleic acid amplification tests for detection of influenza A virus are shown in Table 1. The sensitivities of the Alere and Liat tests were 71.3% (95% CI, 60.5 to 80%) and 100% (95% CI, 95.4 to 100%), respectively. The specificities of both tests were 100% (95% CI, 92.7 to 100%). In this analysis, equivocal results for the FilmArray test were considered positive, since each was confirmed as positive by at least one of the comparators. When the results of Alere and Liat tests were compared, significantly more positive samples were detected by Liat (McNemar test, $P < 0.0001$).

The Alere test was positive for influenza A virus in 39.6% samples from pediatric patients and in 47.3% of samples from adult patients. The Liat test was positive for influenza A virus in 52.8% of samples from pediatric patients and in 68.4% of samples from adults.

The software that supports each of the test systems blocks user access to the cycle thresholds (C_T); however, Roche provided C_T values for those samples positive for influenza A and B viruses from the stored run files on the Liat instrument. The frequency distribution of the C_T values for the 80 samples positive for influenza A virus in the Liat test is shown in Fig. 1. C_T values were rounded to the nearest whole number. We found that the Alere test missed only 2 of 59 (3.4%) positive samples that had Liat C_T values of ≤27 but missed all of 21 (100%) positive samples that had Liat C_T values of ≥28 (Fig. 1). Although the C_T provides only a rough estimate of initial target concentration, it appeared that Liat was better able to detect low-positive samples.

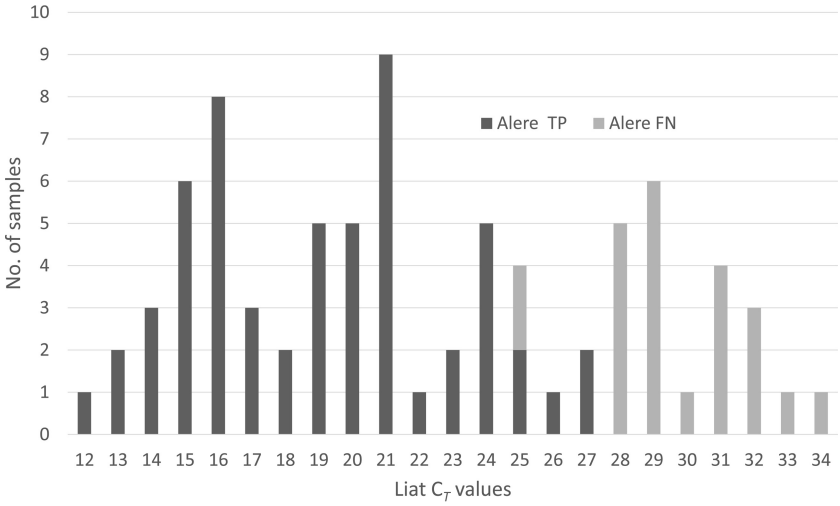

FIG 1 Frequency distribution of cycle threshold (C_T) values for samples positive for influenza A virus in the Liat test. C_T values were rounded to the nearest whole number. True-positive (TP) and false-negative (FN) Alere test results are indicated in black and gray, respectively.

TABLE 2 Comparison of Alere and Liat with the FilmArray test for detection of influenza B virus

Alere or Liat result	No. with FilmArray result:	
	Positive	Negative
Alere		
Positive	15	0
Negative	1	113
Liat		
Positive	16	0
Negative	0	113

In addition, 12 residual false-negative Alere influenza A virus samples were tested with the quantitative real-time RT-PCR influenza A virus assay. The copies per milliliter in the transport medium ranged from 10 to 80,000 (mean, 10,900; median 1,700). When the concentration of virus was adjusted to copies per Alere reaction using the dilution of transport medium in the elution buffer and 100 μl test input volume, all but the one sample with the highest viral load were below the limit of detection of the assay as described in the package insert (data not shown).

The results of the three nucleic acid amplification tests for detection of influenza B virus are shown in Table 2. The sensitivities of Alere and Liat were 93.8% (95% CI, 71.7 to 98.9%) and 100% (95% CI, 80.6 to 100%), respectively. The specificities of both tests were 100% (95% CI, 96.7 to 100). The single positive sample not detected by the Alere test had the highest Liat C_T of 32. The Alere and Liat tests performed comparably to each other and to the FilmArray test for detection of influenza B virus.

Invalid Liat test results were initially obtained with 2 samples (1.6% failure rate). On repeat testing, 1 was positive for influenza A virus and 1 was negative for both viruses. No invalid test results were obtained with the Alere test.

Three samples used in the study were types not included in the intended use of the three tests compared: one BAL fluid sample, which was positive for influenza A virus in the Liat and FilmArray tests but negative in the Alere test, and 2 nasal aspirates, 1 that was positive for influenza A virus and 1 that was positive for influenza B virus in all three tests.

Other viruses were detected by the FilmArray test in 11 (13.6%) of the samples that were positive for influenza A virus, including 4 with coronaviruses (3 HKU1 and 1 OC43), 3 with rhinovirus/enterovirus, 2 with adenovirus, and 2 with respiratory syncytial virus (RSV). Only one coinfection (6.3%) was detected in patients with influenza B virus, and it was with RSV. In the 33 samples that were negative for both influenza A and B viruses by FilmArray, another virus was detected in 8 (24.2%), including 4 coronaviruses (2 OC43 and 2 NL63), 2 adenoviruses, 1 metapneumovirus, and 1 RSV.

DISCUSSION

Although both the Alere and Liat tests were designed to be used as point-of-care tests, they differ in several important features. The Alere test requires multiple components and steps and a wait time of 6 min while the sample receiver heats before the analysis begins. The operator can then leave the instrument unattended. The Liat test has fewer steps, which can be completed by the operator in less than 1 min, after which time the instrument can be left unattended. The CLIA complexity of the Alere test is considered moderate if performed from swab specimens in transport medium, whereas the Liat is considered waived for the same specimen type. The use of swab specimens in transport medium gives clinicians and laboratorians more flexibility for viral testing. Ease of operation, test complexity, and flexibility are important factors when considering which test to deploy in near-patient locations.

In the Alere test, 200 μl of transport medium is first added to the sample receiver, which contains 2.5 ml of elution buffer prior to analysis. Two 100-μl aliquots of the diluted sample are then transferred to the test base. In contrast, 200 μl of transport medium is added directly to the Liat tube without prior dilution for analysis. Three hundred microliters of transport medium is added directly to the FilmArray pouch. The additional dilution may have contributed to the poor sensitivity of the Alere test that we observed with specimens in transport medium.

Another difference between the two point-of-care tests is that only the Liat test extracts and purifies the nucleic acids from specimens prior to analysis. This may make the Liat test more robust and less likely to be influenced by nucleic acid degradation that may occur in specimens that are frozen and thawed and by the presence of amplification inhibitors. The lab-based FilmArray pouch also extracts and purifies the nucleic acids before analysis.

A limitation of our study is that testing with both Alere and Liat was performed with samples that had been through one freeze-thaw cycle. Although we were assured by Alere that a single freeze-thaw cycle should not negatively impact the performance of the test, the package insert does not list freezing as an option for storing transport medium prior to testing. Freezing at −70°C is an option given in the Liat package insert for long-term storage for samples. Another limitation of our study is that it was performed in a clinical laboratory under controlled conditions by a single experienced medical technologist and, consequently, may represent a best-case scenario for the performance of tests designed to be done in near-patient settings by nonlaboratory personnel.

Although the number of samples tested was not large, the sample size was sufficient to demonstrate a significant difference in the performances of the Alere and Liat tests for detection of influenza A virus. However, the assays had similar performance characteristics for detection of influenza B virus, although the number of positive specimens for influenza B virus was much smaller.

We found a lower percentage of positive samples for influenza A virus obtained from pediatric patients than for that from adult patients. This most likely was a consequence of greater use of rapid influenza virus antigen testing in our pediatric outpatient locations, with the FilmArray performed often on patients who are antigen negative. Although rapid influenza virus antigen testing is available in our adult emergency department and in many clinics, the physicians in these locations rely more heavily on the FilmArray as a primary test because of the poorer sensitivity of rapid antigen tests in adults.

The sensitivity of the Alere test of 71.3% for detection of influenza A virus that we observed was lower than that reported by others (6–9). Three of these previous reports compared it to the Xpert Flu A/B (Cepheid, Sunnyvale, CA) assay (7–9), and one compared it to the FilmArray assay (6) as we did. All of the previous studies also used respiratory samples in transport media and samples that had been frozen prior to testing, which was similar to our protocol. The difference may be explained by our study set, which was enriched for low-positive samples, many of which were

below the limit of detection of the Alere test. This could be due to patients presenting to our clinics later in the course of their illness, the inclusion of a large number of samples from adults, and the use of rapid influenza virus antigen tests as the primary screening test in our pediatric population.

Our results with the Liat test were similar to the previous reports of its performance characteristics compared to those of other commercially available nucleic acid amplification tests for detection of influenza A and B viruses, the Prodesse ProFlu+ (Hologic, San Diego, CA) and Simplexa Flu A/B and RSV Direct (Focus Diagnostics, Cypress, CA) assays (10, 11). We found that it was a robust platform for point-of-care diagnosis of influenza that provided results comparable to those of the laboratory-based FilmArray test in our patient population.

Coinfections were detected by the FilmArray test in 13.6% of patients with influenza A virus infections and in 6.3% of patients with influenza B virus infections. These coinfections would have been missed if either the Alere or Liat test had been used as the primary test for influenza A and B, since it is unlikely that FilmArray would have been ordered in these cases. Although the association between coinfections and severity of disease remains unclear, three of these coinfections involved RSV, a virus that may significantly impact treatment and management decisions (13).

The availability of CLIA-waived rapid nucleic acid amplification tests holds promise to significantly improve near-patient diagnostic testing for influenza and may facilitate changes in how these patients are managed clinically. The increased diagnostic yield over rapid influenza virus antigen detection tests could range from 10 to 30% (4, 14), but direct comparisons of the performance characteristics of these tests are lacking. Future studies should focus on establishing the true increase in diagnostic yield and the clinical and financial impacts of implementing point-of-care molecular diagnostics for influenza.

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